location from Blimp1 staining. However, this study provides the first rough description of PGC migration in *M. domestica* and serves as a foundation for further experiments.

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Program/Abstract # 292
A molecular dynamics study on the Tre1 G protein-coupled receptor
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The Tre1 G protein-coupled receptor is required for proper germ cell migration in *Drosophila melanogaster*. In a severe partial loss-of-function alleles of tre1, and tre1 sctt, the germ cells scatter across the posterior half of the embryo rather than forming two gonads. The molecular lesion in tre1 sctt is a point mutation that results in an in-frame deletion of eight amino acids, RYILIACH, which is located at the junction of the third transmembrane domain and second intracellular loop. The highly conserved arginine within this deleted region is critical for Tre1 function. However, it is not known whether the loss of these amino acids affect Tre1's structure. The working hypothesis is that the amino acids RYILIACH are required to keep Tre1 in a fully functional conformation. As there is no crystal structure of Tre1 available, homology modeling of both wild-type Tre1 and Tre1 sctt was performed using the I-TASSER platform to generate three-dimensional structure predictions. These models have been further refined through the use of molecular dynamics simulations with the NAMD simulation package.

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Program/Abstract # 293
A crucial role for lipid phosphorylation in WntD-mediated primordial germ cell migration
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Although much work has been done characterizing genes controlling primordial germ cell (PGC) migration in *Drosophila* embryos, a major unanswered question is the identity of molecules providing guidance cues to these cells. We have previously demonstrated that the ligand WntD utilizes a β-catenin-independent pathway to control PGC migration, leading us to hypothesize that this novel signaling pathway could reveal insights into the mechanism of PGC guidance. We therefore undertook a suppressor screen to identify components of the WntD signaling pathway and discovered that loss of either CG16708, a putative ceramide kinase, or CG31873, a putative multi-substrate lipid kinase, suppresses WntD overexpression. Additionally, embryos double homozygous mutant for both kinase genes display a WntD mutant-like phenotype in primordial germ cell migration. We hypothesize that the WntD signaling pathway produces a phospholipid substrate that can be shaped into a gradient by phospholipid phosphatases Wun and Wun2, thus providing directional cues to migrating PGC.

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Program/Abstract # 294
***Xenopus* Nanos1 is required to preserve PGCs from endoderm specification**
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A significant problem in development is how germ cell fate with its characteristics of totipotency is preserved in the context of somatic cell differentiation. Nanos is expressed in multipotent cells, stem cells, and primordial germ cells (PGCs) of organisms as diverse as jellyfish and humans. The only molecular role assigned to Nanos is as part of a translational repression complex with Pumilio. Here we show by loss-of-function experiments that *Xenopus* Nanos1 is required for PGC preservation. Knockdown of maternal Nanos1 resulted in a significant decrease in PGCs and loss of germ cells from the gonads. Nanos1 mutant embryos were rescued by co-injection of Nanos1 message, indicating the specificity of the morpholino. PGCs deficient in Nanos1 inappropriately express somatic genes such as Xsox17-alpha and Bix4, essential for endoderm specification. Furthermore, whereas normal PGCs do not become transcriptionally active until neurula, Nanos1 depleted PGCs express Xsox17-alpha by stage 10, similar to when somatic endoderm initiates their expression. Consistent with this premature gene transcription, PGCs now express a hyperphosphorylated RNA Pol II-CTD. Lineage tracing and TUNEL staining revealed that Nanos1 deficient PGCs fail to migrate out of the endoderm. They appear to undergo apoptosis rather than convert to normal endoderm. We propose that Nanos1 functions to translationally repress RNAs that normally specify endoderm and promote apoptosis, thus preserving the germline. This work was supported by the NIH grant GM33932 to MLK.

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Program/Abstract # 295
Oskar predates the evolution of insect germ plasm
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Oskar is the only gene known to be both necessary and sufficient for germ cell specification in *Drosophila melanogaster*. However, despite this essential function, oskar has thus far only been found in the genomes of holometabolous insects that specify their germ line through the inheritance of a specialized cytoplasm termed "germ plasm." Using high-throughput transcriptome sequencing, we have identified an ortholog of oskar from the cricket *Gryllus bimaculatus*, a hemimetabolous insect that is thought to retain ancestral characteristics of insect oogenesis and embryogenesis. Like all crickets and grasshoppers, *Gryllus* lacks germ plasm and appears to specify its germ cells inductively during mid-embryogenesis. Gb-oskar is expressed at high levels in ovaries, consistent with a conserved function in oogenesis, but does not localize within oocytes or to developing germ cells in embryos. We are currently working to determine the function of Gb-oskar using RNAi. The study of oskar from a basally-branching insect will provide insight into the evolutionary origins of this gene, and may shed light on the evolution of germ plasm in insects.

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Program/Abstract # 296
Ultrastructure of putative germ plasm in penaeid shrimp
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There is controversy about the source of the germ cells in penaeid shrimp. Histological studies in *Penaeus kerathurus* and other species suggest that the primordial germ cell forms during gastrulation from a descendant of the ventral mesendoblast and can be identified by a large nucleus relative to the cytoplasm and distinctive chromatin. In contrast, a recent study of vasa mRNA localization in *P. chinensis* suggests that the germ cells form at the later limb bud stage, from five cell clusters at the base of the limbs and cephalic lobe. Supporting the first hypothesis, nucleic acid fluorescence staining has identified a putative germ granule (termed an intracellular body or ICB) in several penaeid shrimp species, which is inherited by one of the mesendoblasts. Sytox Green, a general nucleic acid stain, and SYTO RNASelect, an RNA-preferential stain, both label the ICB but DNA-preferential stains such as DAPI do not. In addition, the staining of the ICB can be removed by RNase but not DNase treatment. To determine if typical characteristics of germ plasm could be found at the ultrastructural level, *P. japonicus* embryos were examined by transmission electron microscopy during cleavage and gastrulation. ICBs from cleavage stage embryos were composed of granular, membraneous material distinct from the surrounding cytoplasm, which was enriched with mitochondria. This appearance was retained through the 62-cell stage, at which time the mesendoblasts had become internalized. At the late gastrula stage, the hypothesized primordial germ cell was identified but there was no evidence of the ICB. We conclude that the shrimp ICB region has ultrastructural characteristics commonly seen in germ plasm in other species.

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Program/Abstract # 297

Localization of the Vasa homolog and formation of germ granules during oogenesis of the sea urchin *Strongylocentrotus intermedius*

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In sea urchins, germline segregation occurs during embryogenesis by selective accumulation of germline-specific markers in the small micromere lineage, which descendants contribute to gamete production in adults. However, the accumulation and distribution of germline determinants during oogenesis and the role of maternal contribution to germline formation in sea urchin embryogenesis are still poorly known. Vasa is widely used as a germline marker and is a component of unique ribonucleoprotein complexes known as nuage, chromatoid bodies, and germ granules that are essential for gametogenesis and germline segregation in embryos. Despite the presence of Vasa in the sea urchin eggs, germ granules were not detected until micromere segregation occurred. Therefore, in this study, we analyzed the distribution of the sea urchin homolog of a Vasa protein during oogenesis of the sea urchin *Strongylocentrotus intermedius* and also studied its intracellular localization. We found that Vasa localized in the cytoplasm of all germ cells and eggs and had two distinct intracellular granular patterns. In mitotic germ cells, oogonia, and primary oocytes, Vasa often localized in perinuclear nuage-like structures. In eggs, Vasa was detected only in the cortical layer; this pattern was initiated in the full-grown primary oocytes that had lost cortical restriction during the first embryonic cleavage. These preliminary results show that the Vasa is not only accumulated during sea urchin oogenesis, but also is incorporated in maternal germ granules associated with the egg cortex. Supported by FEB RAS grant 09-I-P22-04.

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Program/Abstract # 298

***Cyp26b1* regulates sex-specific timing of meiotic initiation independent of retinoic acid**

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Entry into meiosis was initially proposed to be an intrinsic property of fetal germ cells unless prevented by a meiosis-inhibiting factor produced in the testis. Other studies suggested that sex-specific initiation of meiosis requires retinoic acid (RA) synthesized by Raldh2 in the mesonephros for induction of Stra8 in fetal ovary, with expression of the RA-degrading enzyme Cyp26b1 in testis delaying meiosis until postnatally. Here, investigation of Raldh2−/− mice lacking RA synthesis in mesonephros indicated that Stra8 expression in the ovary does not require RA signaling; meiotic markers Scp3 and g-H2AX were also expressed normally. We also found that Stra8 is expressed in the absence of physiologically detectable levels of RA in either mesonephros or gonad using the RARE-lacZ transgene which was validated to be a sensitive reporter for physiological levels of RA (25 nM). Chromatin immunoprecipitation studies demonstrated that RARs do not bind efficiently to a putative RARE upstream of Stra8. In addition, ketoconazole inhibition of Cyp26b1 in Raldh2−/− testis allows RA-independent induction of Stra8, but only when the mesonephros remains attached, pointing to a non-RA signal from the mesonephros that induces Stra8 in the adjacent gonad. These findings demonstrate that Cyp26b1 prevents the onset of meiosis by metabolizing a substrate other than RA which regulates Stra8 expression.

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Program/Abstract # 299

The role of Geminin in germinal stem cells

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Stem cells preserve tissue stability by balancing self-renewal with differentiation into somatic cells. The unstable regulatory protein Geminin is one of the factors that determine if stem cells will continue to divide or terminally differentiate. Geminin is required for maintenance of embryonic stem cells and is thought to maintain cells in an undifferentiated state while they proliferate. Geminin is a bifunctional protein. It prevents a second round of DNA replication during S and G2 phases by inhibiting the essential replication factor Cdt1. It also inhibits cell differentiation by binding to and inhibiting several transcription factors, including members of the Homeobox family and the retinal development protein Six3. To test this model, we have developed a mouse model in which Geminin is specifically deleted from germ cells. This mouse carries a loxP-flanked Geminin allele and expresses Cre-recombinase under the control of the germ cell-specific Vasa promoter. We find that Vasa-Cre+/GemininloxP/loxP males are viable but not fertile. The number of germ cells in Vasa-Cre+/GemininloxP/loxP mice is normal at birth but they are lost within the first week of life. Geminin is not required for meiosis and spermiogenesis. Our results indicate that the defect arises in the proliferative phase of spermatogenesis. We are now testing whether the spermatogonial defect is caused by replication abnormalities or by a change in the spermatogonial transcription pattern.

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